

peptide disclosed in that reference. In response, claim 1 is hereby canceled and is not reinstated in its "open" form. Further, claims 3 and 4 now depend from "closed" claim 23, rendering this rejection moot.

The Examiner has rejected claims 1, 3-6, 17, 18, 21 and 22 under 35 U.S.C. §102(b) for purported anticipation by Canadian Patent No. 2,072,009 ("the '009 patent"). Specifically, the Canadian patent discloses a partial sequence of an undefined peptide fragment of the *Borrelia burgdorferi* pC protein. The partial sequence has five consecutive amino acids in common with one species of the stress protein fragments claimed in the present application (amino acids 91-95 of *M. tuberculosis* HSP60). More specifically, the pertinent amino acid sequences read (identifying the common amino acid residues by the lines connecting the two sequences):

A. Claimed embodiment	G-D-G-T-T-A-T-V-L-A-Q-A
(species)	
B. pC sequence	K-I-Y-D-S-N-A-T-V-L-A-V-K

For the following reasons, the disclosure in the '009 patent of the partial pC sequence does not anticipate or render obvious the claims of the present application.

1. The prior art sequence does not meet the limitation of the claims of the present application.

Independent claim 23 requires that the peptide of 7-30 amino acids has the sequence of a part of the amino acid sequence of a microbial protein having a conserved mammalian stress protein homologue. This is not the case with the prior art pC protein. Applicants know of no mammalian counterpart to the B.

*burgdorferi* pC protein. Further, no peptide which can be derived from the disclosed pC partial amino acid sequence has 7-30 amino acids which are identical to 7-30 amino acids of a microbial protein having a mammalian stress protein homologue.

Second, the claims also require that the peptide has a T-cell epitope corresponding to a T-cell epitope of the mammalian homologue. There is no indication that the pC sequence is a T-cell epitope. As discussed in earlier papers, a T-cell epitope has at least 7 amino acids. The two amino acids flanking each side of the ATVLA core sequence in pC are not the same as the two amino acids flanking the same core sequence in the *M. tuberculosis* protein or in its mammalian counterpart. As such, it cannot be said with any certainty that any 7-30 mers which can be generated from the disclosed pC amino acid sequence include T-cell epitopes, let alone T-cell epitopes with the same specificity as the claimed peptides.

No 7-30 mer which can be derived from the pC partial sequence is the same as any known microbial protein sequence having conserved mammalian stress protein homologue. Further, there is no indication of, and no predictability thereof, that the pC-derived 7-30 mer having the core ATVLA sequence is a T-cell epitope, or a T-cell epitope having the same specificity as the claimed epitopes. Therefore, the disclosure of the partial sequence of pC in the '009 patent does not anticipate the claimed peptides.

2. No 7-30 mer peptide, corresponding to the partial amino acid sequences of pC disclosed in the '009 patent, ever existed or has been suggested.

In the '009 patent, the nucleic acid sequence of the *B. burgdorferi* pC protein was determined in the following manner, disclosed on pages 20-26 of that patent. First, a plasmid library of *B. burgdorferi* DNA was created in *E. coli* cells. Second, the pC protein was isolated by Western Blot and trypsinized. At least two trypsinized fragments of pC, of unknown size, were purified by HPLC and were sequenced by an N-terminal protein sequencing method. The partial amino acid sequence of one fragment (pC-p1, disclosed on page 26 of the '009 patent) was determined. Degenerate oligonucleotides were generated from the partial amino acid sequence and the genomic clone of pC was identified in the *E. coli* library.

As a preliminary matter, because the protein sequence of the pC fragment was determined by an N-terminal method, the pC-p1 13 mer never existed in solution. One page 23, lines 34-37 of the '009 patent, it is stated that two of the HPLC-purified fragments were partially sequenced (resulting in the disclosed pC-p1 sequence). In N-terminal sequencing, the N-terminal amino acids are removed sequentially to determine the sequence of the peptide. Because there is no indication of the size of the HPLC-purified un-sequenced peptide fragment, there is no evidence that the un-sequenced or partially sequenced peptide fragment (the remainder of the C-terminal portion of the peptide fragment) is a 7-30 mer. For instance, if the purified peptide fragment was 50 amino acids, only 6 amino acid residues could be removed and leave the ATVLA core sequence intact. As such, the minimum size of the peptide having the ATVLA core sequence is 44 amino acids. Lastly, the pC-p1 13 amino acid sequence never existed as a

contiguous peptide, but existed only as a record of which individual peptides were thought to be severed from the N-terminal portion of the purified pC peptide fragment. Therefore, there is no evidence that any pC peptide fragment ever existed which meets the metes and bounds of the claims of the present application.

With respect to the disclosure of the pC-p1 peptide sequence, this sequence is not anticipatory. It is a partial sequence of a peptide fragment of unknown size. It does not teach or suggest the isolated 13 mer or preparation of the 13-mer for any reason. As outlined in further detail in Applicants' Supplemental Preliminary Amendment, dated May 25, 1999, no skilled artisan would refer to the pC-p1 sequence as indicative of the peptide sequence of pC, due to the contradiction between that sequence and the sequences derived from the genomic clone.

The Examiner provides two possible reasons why the full length sequence was not identical to the pC-p1 peptide. First, the Examiner states that the sequence of the genomic clone may have been erroneously determined. However, the probability of an error changing the Thr codon (ACN) to a Phe codon (TT[T/C]) is  $(1-5\%)^2$  (the likelihood of two sequential nucleotide sequencing errors). The Examiner does not provide the likelihood of a protein sequencing error which could have occurred in the partial N-terminal sequencing of the HPLC-purified pC fragment. Nor does the Examiner account for the effect of the purification and trypsinization digestion processes, to which the sequenced peptide was exposed, on the determined protein sequence. Lastly, to a researcher, the accuracy of a published nucleotide and amino

acid sequence of a genomic clone is much more important than the accuracy of a peptide sequencing protocol used to identify degenerate probes for identification of the genomic clone. So long as the partial peptide sequence is reasonably accurate, the large, 40 mer degenerate probe used to identify the genomic clone would stand a very good chance of hybridizing with a corresponding genomic clone, despite a few mismatched amino acids. It is more likely that more care (i.e., repeated sequencing experiments) was taken to produce the sequence of the genomic clone. Therefore, it is more likely that the sequence of the genomic clone is more accurate than the sequence of the peptide.

The Examiner also states that Thr and Phe are conservative substitutions, and therefore, strain variants might account for the difference between the initial partial protein sequence and the amino acid sequence of the genomic clone. First, this is unlikely because the same *B. burgdorferi* strain was used to prepare the genomic library and the peptide which was partially sequenced to determine the pC-p1 sequence. Second, Thr and Phe are far from conserved. The side chain of Thr is a polar ethanol group. The side chain of Phe is a non-polar phenyl group. These are not conserved amino acids and one could not expect the proposed variants to occur because the substitution of an amino acid having a bulky aromatic side chain for an amino acid having a small polar side chain would be expected to affect the biological function of a relatively small, 212 amino acid protein.

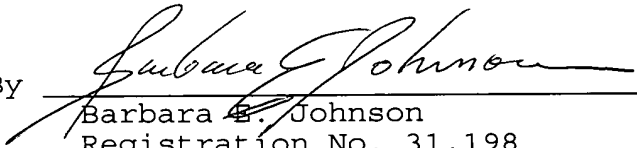
In sum, the sequence disclosed in the '009 patent does not anticipate the sequences claimed in the present invention. No microbial protein having a mammalian stress protein homologue has a 7-30 mer identical to the partial epitope sequence disclosed in the '009 patent which meets the requirements of any claim of this application. Further, there is no reason to believe that the partial peptide sequence disclosed in the '009 patent harbors a T-cell epitope, especially one identical to those claimed. Lastly, there is no reason to believe that the partial peptide disclosed in the '009 patent ever existed except as an experimental or typographical error. In any case, there is no evidence on record that the asserted 13 mer ever physically existed or was contemplated to exist as a part of a 7-30 mer. For this reason, Applicants respectfully request reconsideration of the rejection of claims 1, 3-6, 17, 18, 21 and 22, now claims 3-6, 18, 22 and 23, for anticipation by the '009 patent.

For the reasons stated above, claims 3-6, 18, 22 and 23 define over the prior art of record and are in proper form for allowance. Applicants respectfully request reconsideration of the rejections set forth in the Office Action, dated August 2, 1999, and allowance of claims 3-6, 18, 22 and 23.

Respectfully submitted,

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